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II. *On the Origin of Parasitism in Fungi.*

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RESPECTING the various modes by which parasitic fungi gain access to the interior of the host-plant, much is known. DE BARY (1) demonstrated that the germ-tubes of æcidiospores and uredospores enter solely through the stomata, whereas germ-tubes of teleutospores, and also those of various other parasites, enter by piercing the walls of the epidermal cells, or of the guard-cells of the stomata. Other fungi gain an entrance sometimes by a stoma, sometimes by piercing the wall of an epidermal cell.

The same author also observed that the zoospores of *Cystopus* and of *Peronospora umbelliferarum*, when deposited on the leaf of a suitable host-plant, germinate and the germ-tube enters a stoma, whereas when germination takes place in water the germ-tubes soon die. MARSHALL WARD has shown (2) that in the case of a species of *Botrytis* an entrance into the host-plant through the cell-walls of the epidermis is effected by means of the secretion of a ferment by the tip of the germ-tube, whereby the substance of the cell-wall is softened.

On the other hand, but little has been accomplished bearing on the question as to why certain plants are attacked by parasitic fungi, and what has been done turns mainly on supposed special susceptibility, predisposition to disease, or the gradual lowering of the constitution due to cultivation, &c., of the plants attacked.

Such statements, however, even if true in a general sense, do not indicate the specific reason why a parasitic fungus possesses the power of gaining an entrance into the tissues of one particular kind of plant only. Nevertheless, such is well known to be the case, for although the spores of a parasitic fungus will germinate on the surface of the leaf of any plant if it is damp, yet the germ-tubes will only enter the tissues and infect the particular kind of plant on which the fungus is known to be parasitic.

This condition of things suggests the existence of some attractive agency exercised unconsciously by the host-plant over its parasite. This power of the host-plant over

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its parasite I shall endeavour to prove to be due to what PFEFFER has called "chemotaxis," or the direction of movement due to chemical stimuli.

The idea that chemotaxis may play some part in favouring parasitism has already been suggested by MARSHALL WARD, who, in discussing predisposition and immunity in plants, says (3): "Moreover, as we know from the recent advances in the study of enzymes and of chemotactic substances, of toxins and antitoxins, such substances and conditions as I have postulated above do actually occur in the living cells, and there is not only no absurdity, but, on the contrary, every show of probability that—since the structural features elucidated by the microscope are not responsible for the phenomena of immunity and susceptibility on the part of the host, or of capacity or incapacity to infect on the part of the fungus—it is in the domain of the invisible biological properties of the living cell that we must expect the phenomena to reside."

ISTVANFFI also considers that chemotaxis may prove to be an important factor in connection with parasitism in fungi (4).

At this stage it may be well to briefly indicate the significance of chemotaxis as defined above. The attraction of plasmodia of the Myxogastres towards a nutritive solution placed in their vicinity was described by STAHL (5), and the term "trophotropism" suggested, but it was due to the brilliant investigations of PFEFFER (6) that the force he termed "chemotaxis" was clearly demonstrated. This author experimented with the antherozoids of cryptogams, bacteria, protozoa, &c., and proved that not only food-stuffs, but also solutions of various salts, acids and alkalies possessed the power of directing the movements of the organisms experimented upon. He further showed that certain substances attracted bacteria, &c.—positive chemotaxis—whereas other substances exercised a repelling influence—negative chemotaxis.

Following PFEFFER, MIYOSHI (7) has shown that by placing the epidermis of an onion bulb-scale or a thin film of mica through which very minute holes had been pierced, on the surface of gelatine containing a positively chemotactic substance, as dextrin, plum decoction, sugar, &c., the germ-tubes of fungus spores sown on the surface of the epidermis or mica grew towards and finally passed through the stomata or pierced holes into the nutrient substance. He also demonstrated that the hyphæ of *Penicillium glaucum* and species of *Botrytis* passed through the epidermal cell-walls of living leaves, which had previously been charged with a positively chemotactic substance by hypodermic injection.

MIYOSHI tested the action of a large number of salts, acids, alkalies, &c., on fungi, and gives the result in tabulated form. Among strongly positive chemotactic substances may be mentioned saccharose, glucose, asparagin and peptone; among the less pronounced in this direction are included levulose and lactose. Maltose is generally neutral. Free organic and inorganic acids, alkaloids and certain salts, proved to be negatively chemotactic or repellent.

The same author also caused the germ-tubes of *Penicillium glaucum* to bore through the cells of elder pith, and to pass through the pits of coniferous wood that had been injected with a chemotactic solution (8). A preliminary series of experiments were first conducted with the object of testing the efficacy of chemotaxis on a larger number of fungi belonging to different groups than had previously been done.

As the primary object of the present research was to discover, if possible, whether the entrance of the germ-tubes of parasitic fungi into the living tissues of a plant was directly due to the attraction of some specific substance present in the cell-sap, only those substances occurring normally in living plants were tested. Among these may be enumerated cane sugar (saccharose), grape sugar (glucose), asparagin, malic acid, and the cell-sap of various plants.

These substances were tested respectively in proportions varying from 0·05–10 per cent., along with 6 per cent. of gelatine. Petri dishes were mostly used for the experiments. A thin sheet of mica or of celluloid of the kind used for photographic films 3 centims. square, having a minute hole pierced through the centre, was placed on the nutrient substance in the petri dish. The upper surface of the mica or celluloid film was moistened with sterilised tap water, and the spores to be experimented upon were sown in the water. A piece of damp blotting paper placed on the inside of the cover of the petri dish prevented desiccation of the spores.

The accepted proof of the germ-tubes of a fungus being chemotactically attracted by the substance present in the nutrient substance, consisted in their growth being directed towards the hole in the mica, and eventually passing down through the perforation into the nutrient substance. When the substance proved to be negatively chemotactic, or repellent, the germ-tubes showed no tendency to grow towards the perforation in the mica, or those that happened to grow in its direction altered their course and receded when they approached within the sphere of its influence.

In other experiments the epidermis of an orchid—*Oncidium bellatulum*—was used instead of mica.

It is important to bear in mind that chemotaxis is not in any way connected with germination. Practically all spores, whether belonging to saprophytes or parasites, will germinate in water alone, or in a dilute solution of sugar in water, without, however, its exercising the slightest influence on the direction in which the germ-tubes grow. It is only when a substance proving to be positively or negatively chemotactic as the case may be, is localised at some given point, that growing germ-tubes are attracted or repelled by its presence, when once they approach within the range of its influence.

In experiments conducted by sowing spores in a film of water on a mica or celluloid plate, placed on a medium to be tested as to its chemotactic power, it may be assumed that a small amount of the substance passes up through the perforation

in the mica plate, and slowly diffuses in the water on its surface. If the substance proves to be positively chemotactic to the fungus experimented with, the germ-tubes on coming in contact with the substance diffused in the water, follow the diffused substance, which becomes more concentrated as the perforation in the mica is approached, through which the germ-tubes eventually pass into the yet denser attractive substance in the gelatine.

If the substance proves to be not chemotactic, the germ-tubes grow in various directions, and are not at all influenced by the substance diffused in the film of water on the mica, and do not pass through the perforation into the gelatine below.

This last experiment proves that difference of density in the absence of a chemotactic substance does not enter into the principles involved in chemotaxis, as the germ-tubes do not pass from the film of water on the surface of the mica, into the denser substance below unless specifically attracted by some other factor than that of difference of density.

Finally, to prove that germ-tubes do not pass through minute openings, simply because such openings in the form of stomata exist in the epidermis of leaves, as appears to sometimes have been assumed; a 2-per-cent. solution of sugar in water was placed in a Petri dish, a plate of celluloid perforated with numerous minute holes was floated on the surface of the solution, and its upper surface moistened with a portion of the solution on which it floated. Spores of *Botrytis cinerea* were sown in the liquid on the surface of the mica, and although germination was very vigorous, the germ-tubes did not pass through the perforations in the mica into the solution below.

Miyoshi does not give details of his mode of injecting living leaves. The method I employed is as follows: Living leaves still attached to the plant were injected with the chemotactic solution. An ordinary hypodermic syringe having a very fine needle was used. In a fairly fleshy leaf the needle can, with practice, be inserted for a distance of 2 centims., just under the epidermis of the under surface of the leaf. As the needle is very slowly withdrawn, a slight pressure is applied to the piston; when successfully accomplished, up to 0.5 of a cubic centimetre of the solution can be injected, which betrays its presence externally by a slight discoloration of the epidermis, and extends over an area of 1-2 square centims. The spores are sown in a film of water on the surface of the cuticle, over the injected portion.

The following table shows the positive (+), or negative (−) chemotactic action of various substances, on the germ-tubes of fungi. Those fungi marked with an asterisk (*) are obligate parasites; a dagger (†) indicates facultative parasites; the remainder, so far as is known, are pure saprophytes. A 2-per-cent. solution of the substance to be tested, in 6 per cent. of gelatine was used.

A glance at the table shows that sugar is by far the most general of positive chemotactic substances. The germ-tubes of all the saprophytes, with the exception of

Name of fungus.	Cane sugar.	Grape sugar.	Asparagin.	Malic acid.	Decoction of cucumber leaves.	Decoction of tomato leaves.	Decoction of bramble leaves.
* <i>Cercospora melonis</i> . . .	—	—	—	—	+	—	
* <i>Macrosporium tomato</i> . . .	—	—	—	—	—	+	
* <i>Cladosporium fulvum</i> . . .	—	—	—	—	—	+	
† <i>Botrytis cinerea</i>	+	+	+	—	—	—	
<i>Trichothecium candidum</i> . . .	+	+	—	—	—		
† <i>Cladosporium epiphyllum</i> . . .	+	+	—	...	—		
† <i>Penicillium glaucum</i>	+	+	—	—	—		
† <i>Trichoderma lignorum</i>	+	+	+	...	—		
* <i>Sclerotinia fructigena</i>	+	+	—	+			
(Conidia)							
† <i>Dendryphium comosum</i>	+	+	—	—	+	—	—
† <i>Fusicladium dendriticum</i>	+	—	—	+			
† <i>Mucor racemosus</i>	+	+	—	—	—		
<i>Stysanus stemonites</i>	—	—	—	—	—		
<i>Verticillium lateritium</i>	+	+	—	—			
<i>Torula herbarum</i>	+	+	—	—			
<i>Bispora monilioides</i>	+	+	—	—			
* <i>Phragmadium violaceum</i>	—	—	—	—	—	—	—
(teleutospores)							

Stysanus stemonites, and all the facultative parasites respond strongly to this substance. On the other hand, the action of sugar on the germ-tubes of obligate parasites, although evident, is very slight, and was not sufficiently powerful in any instance to attract the germ-tubes through perforations in mica, or through stomata.

On again referring to the table it will be seen that the only substance exercising a marked positive chemotactic influence over the germ-tubes of obligate parasites, is the cell-sap of the host-plant on which they are parasitic.

This discovery gives us the first glimpse as to why certain fungi are obligate parasites on one species of plant only. It means that the fungus has become so sharply specialised, that its germ-tubes respond alone to a given substance, or to a combination of substances, present only in the cell-sap of one particular species of plant.

When the specialisation of the fungus is not so sharply defined, we find it capable of attacking several allied species of plants; whereas when we come to such facultative parasites as *Botrytis*, which attacks a greater number of plants belonging to widely separated affinities than any other fungus, it is found that the presence of sugar alone in the cell-sap is sufficient to establish parasitism, provided other conditions are favourable.

The reason why *Botrytis* does not become parasitic on every plant containing sugar in its cell-sap, is probably due to the presence of other substances in the cell-sap which are negatively chemotactic to its germ-tubes.

The following experiments support this idea :—

Experiment 1.—When *Botrytis* spores are sown in a decoction of the cell-sap of green apples, the percentage of germination is small, and the germ-tubes do not continue to grow after the third day. When the apple juice along with gelatine, is used as a chemotactic substance, the spores being sown in water on a mica film, its action is negative. The spores will not germinate in a solution of malic acid in water.

Now, although there is sufficient sugar present in the apple cell-sap to constitute a positive chemotactic substance for the *Botrytis* germ-tubes, this action is neutralised by the more powerful negative chemotaxis of the malic acid present. *Botrytis* is not parasitic on green apples in a state of nature.

Experiment 2.—This experiment was conducted after the method described above, the cell-sap of a half-ripe tomato being substituted for that of apple. Germination of *Botrytis* spores was feeble in the cell-sap, and this substance also proved to be negatively chemotactic. On experimenting with oxalic acid, a substance present in tomato fruit, its action proved to be distinctly repellent to the germ-tubes of *Botrytis*. In this instance, therefore, the repellent action of oxalic acid secured the tomato from being attacked by *Botrytis*.

Negative chemotaxis has been shown by ZILBERBERG and ZELIONY (9) to be the cause of immunity against the action of certain virulent bacteria.

Returning to a study of the behaviour of obligate parasites to certain substances normally present in the cell-sap of their respective host-plants, *Sclerotinia fructigena*, the conidial form of which is better known in this country as *Monilia fructigena*, is a well-known destructive parasite on the fruit of apple, pear, plum, cherry, &c.

Experiments conducted with the spores of this fungus, obtained from mummified apples that had been lying on the ground throughout the winter, showed that the germ-tubes are feebly responsive to sugar alone, also to malic acid alone, but are strongly positively chemotactic to a mixture of 1 per cent. of sugar and 1 per cent. of malic acid. On the other hand, if acetic acid be substituted for malic acid, either used above or in combination with sugar, the germ-tubes show no directive response, thus showing that malic acid, a normal constituent of apple cell-sap, is the important agent in favouring the entry of the germ-tubes of *Monilia* into the tissues of apples.

A further experiment proved that the spores of *Sclerotinia fructigena*, obtained from a mummified cherry and a plum respectively, responded only very slightly, or in some instances not at all to malic acid, either used alone or in combination with sugar. At present material is not available for clearing up this apparent anomaly. It may possibly signify that there are specialised or biological forms of this widely distributed parasite, occurring on apple, cherry, plum, almond, and other fruits, and that each biologic form responds only to some specific substance present in its own particular host.

This idea agrees with the observations of ERIKSSON (10), MARSHALL WARD (3C), and SALMON (11), that biological forms of species of *Puccinia* and *Erysiphe* are restricted to special hosts or even, in some instances, to forms or varieties of a species of host-plant.

The remarks of MARSHALL WARD (3D), respecting the non-adaptability of Uredo-spores to certain species of *Bromus*, appear to bear on the above subject. He says: "In these cases it seems that the infecting tubes reach and penetrate the stomata but are unable to form a mycelium in the tissues, evidently because the living cells are really resistant. How the latter prevent the hyphæ from putting haustoria into their cell cavities is not yet clear, but it looks as if they exerted some deleterious influence on the delicate tips, and thus brought their efforts to abortion."

The answer suggested by the experiments described in this paper is that the non-adaptability was due to the action of negative chemotaxis.

Cercospora melonis is a very destructive parasite, met with only on species of *Cucumis*. The germ-tubes respond chemotactically only to a solution of the host-plant. Experiments seem to indicate that the enzyme called pectase, which is present in some considerable quantity in cucumber leaves, is the specific chemotactic substance in this instance. On the addition of a watery solution of pectine to the extract of cucumber leaves, the chemotactic property of the sap is destroyed, probably due to the coagulation of the pectine by the pectase. Boiling the expressed juice of cucumber leaves, or the addition of a trace of acetic acid also destroy its positive chemotactic influence over the germ-tubes of *Cercospora*.

Pectase is most abundant in leaves that have grown rapidly, hence the greater susceptibility of cucumber plants that are much "forced" over those growing under more normal conditions.

Teleutospores of *Phragmidium violaceum* germinated in water, also in the expressed juice of bramble leaves, the natural host-plant of this obligate parasite. Some time was spent in endeavouring to discover a positive chemotactic substance for the germ-tubes, but without success, the cell-sap of the host-plant even proving to be inert. Finally it occurred to me that the germ-tubes of teleutospores are not infection tubes, but promycelial tubes, which soon produce a few wind-borne promycelium spores. It is the latter spores that directly enter the tissues and infect the host-plant, but I have not had an opportunity of testing the effect of bramble leaf cell-sap on promycelium spores.

In using the expressed juice of leaves for experimental purposes, percentages as used in the ordinary sense cannot be given, but I have adopted the following standard:—Fourteen grammes of the blade of the leaf are thoroughly crushed in a mortar, along with 4 c.c. of sterilised water. The pounded mass is squeezed through muslin, and the liquid product constitutes the stock solution.

This solution is only effective when used immediately after preparation. Boiling destroys its chemotactic property, and the addition of a trace of acetic acid, added

with the idea of checking the development of bacteria, also rendered it neutral to germ-tubes.

A 2-per-cent. of the stock solution prepared from cucumber leaves proved strongly positively chemotactic to the germ-tubes of *Cercospora melonis*, whereas a 5-per-cent. proved as decidedly negative.

Professor MARSHALL WARD has kindly pointed out to me that the expressed juice of a leaf is a very different thing from the cell-sap of the same leaf. Expressed juice is certainly an unknown quantity, and unfortunately the same may be said of cell-sap to a great extent. I have not always been able to detect the exact substance or combination of substances present in the cell-sap, which determines its chemotactic property. In most instances the components of the cell-sap are unknown, and even where they are known are difficult to procure. This difficulty will, however, in all probability be eventually overcome.

At present, however, it is something to know that when a positive chemotactic substance is present in leaf extract, its efficacy is not obliterated if used when quite fresh.

The spores of *Cercospora melonis* germinate freely in 3-per-cent. gelatine and 2-per-cent. sugar, but the germ-tubes show not the slightest directive influence, whereas if the proper amount of cucumber-leaf decoction is added, the germ-tubes are strongly attracted. The same is true of the germ-tubes of other parasites.

MIYOSHI (7A) found that the germ-tubes of *Uredo linearis* were attracted by a decoction of wheat leaves, the host-plant of this parasite, and further demonstrated that the germ-tubes of this fungus, when sown on the epidermis of a leaf of *Tradescantia discolor* that had been injected with a decoction of wheat leaves, passed through the stomata into the tissues of the leaf.

RAY (12) in attempting to convert such sharply defined obligate parasites as species of the Uredineæ and the Ustilagineæ to a saprophytic condition, discovered that to accomplish this object, the fungi had first to be cultivated in a decoction of the host-plant on which they were respectively parasitic, and replacing this nutrient medium by degrees with decoctions of plants less and less allied to the original host.

The experiments described I consider to point to the conclusion that parasitic fungi, and more especially obligate parasites, are highly specialised forms, whose germ-tubes have gradually acquired the property of responding chemotactically to one specific substance only, other than sugar, and even the last-named substance, as previously stated, also plays a certain part in the work of enabling the fungus to enter its host; possibly this part may be connected with the nutrition of the germ-tubes. In the case of the less highly specialised facultative parasites, it would appear that sugar alone is sufficient to enable the germ-tubes to enter the tissues of a plant, unless its power is neutralised by the presence of a more potent negatively chemotactic substance.

It is generally considered that infection of plants by parasitic fungi occurs most frequently during the night in a state of nature. The following experiment supports this idea :—

A batch of vegetable marrow plants growing in my garden last year were badly attached by *Sphaerotheca humuli*. Twelve young leaves showing no trace of the disease were selected for experiment. Six leaves were protected during the day (6 A.M. to 6 P.M.) in paper bags, and left exposed during the night. Six other leaves were enclosed in bags during the night (6 P.M. to 6 A.M.) and exposed during the day. This arrangement was continued for a week; those leaves that had been exposed during the night only were white with the mildew at the termination of the experiment, whereas those leaves that had been exposed during the day only were free from the disease, with the exception of a few very small patches on three of the leaves.

Some of the causes favouring infection during the night are as follows : (1) The presence of a film of moisture on the surface of leaves, &c., an indispensable factor. (2) A greater amount of sugar in the cell-sap. (3) Perhaps a greater amount of those special substances which prove positively chemotactic to the germ-tubes of the parasite; on this point, however, I can offer no direct evidence.

The above conditions collectively tend to produce temporarily, in the plant growing in the open, the “soft” foliage of the gardener, a condition which all experience has proved to be highly susceptible to disease.

In plants grown under glass, more especially when “forced,” the conditions favouring “soft” foliage are constantly at work; which in the aggregate mean the loss of balance between work done by leaves and root respectively. Hence the reason why plants grown under glass are so much more susceptible to disease, than are plants of the same kind grown out of doors.

MARSHALL WARD points out (3E), that if a period favourable to growth of plant life is followed by a fall of temperature, rain, and obscured light, transpiration is lowered and the plant becomes more suffused with water, hence turgescence is promoted. Under such conditions the absorption of oxygen is decreased, and not present in sufficient quantity to complete the oxidations and decompose the organic acids, which accumulate in the cells; such bodies accompanied by glucose in solution, predispose a plant to disease.

Speaking of the potato disease, the last quoted author says (3F.)—“Suppose we take a potato plant the leaves of which are very slightly marked with disease spots, and divide it into two halves as exactly alike as possible, and place each half in a tumbler of water; the two tumblers with their half-plants, are then placed in an ordinary room, side by side, at a temperature of about 20° C., and one is covered close with a bell-jar, and the other left uncovered. In a short time—often a few hours—the covered leaves become black and rotten with the disease; whereas the

uncovered one will go on looking fresh for several days, though it also succumbs at once if covered.

“The question arises whether the rapid spread of the fungus and the rot it causes here are simply owing to the increased supply of water, as the tissues become turgid in the saturated atmosphere under the bell-jar ; or whether we have not here again, in addition, a case where the diminished access of oxygen to the interior of the tissues of the host results in an accumulation of organic acids and other substances which make the excessively turgid cells and thin watery cell-walls more than usually easy prey to the parasite.”

The researches of PFEFFER, MIYOSHI, also my own investigations bearing on chemotaxis, prove that in some instances a very small percentage of a chemotactic substance is sufficient to clearly indicate its positive or negative property. Thus 0·01 per cent. of most organic acids and alkalies is decidedly negative to the germ-tubes of many fungi, whereas the same percentage of cane sugar is as markedly positive in its action. Many substances, as sugar and asparagin, exhibit distinctly positive chemotropic properties in low dilutions, whereas the same substances in higher dilutions, exhibit negative chemotaxis.

It is possible that the prime factor in determining the presence or absence of an epidemic caused by fungi, may not depend so much upon the superabundance of fungus spores in the first instance, as upon the exact amount of chemotactic substance present in the cell-sap of the host-plant ; assuming that the amount of such substance is variable, even within very narrow limits, under varied climatic or other conditions.

A further corroboration of the view that parasitism on the part of fungi, is determined by the presence of some attractive substance in the cell-sap, was furnished by experiments made on an immune cucumber plant. Up to the present, the conception of an immune plant is an individual that resists the attacks of a given parasite, without, however, being able to assign a definite reason as to why ; and even in the most virulent epidemics, it is not unusual to find certain individuals possessing this property of immunity.

The plant experimented upon was one of a batch of ten, brought to Kew by a market gardener from Waltham Cross, whose plants had suffered severely from an epidemic during the previous season. All the plants were supposed to be attacked by a fungus *Dendryphinum comosum*, and were placed in an experimental house for future observation. One of the plants, however, proved to be free from disease, and although surrounded by diseased plants for months, this plant resisted inoculation, both from natural and artificial sources.

Numerous other cucumber plants were successfully infected with spores from the same gatherings as those experimented with on the immune plant. On sowing spores on mica films, in a 2-per-cent. infusion of the cell-sap of the immune plant, I was surprised to find that the percentage of spores that germinated was small, and

that the germ-tubes did not respond chemotactically to the nutrient substance. Numerous further experiments were made with dilutions of the cell-sap of the immune plant, varying from 0·05–5 per cent., and under different temperatures ranging from 50–80° F., but the percentage of germination was in all instances low, and the germ-tubes absolutely refused to be influenced in their direction by the cell-sap.

Check experiments with spores sown on mica films, in contact with a 2-per-cent. solution of cell-sap, obtained from the leaves of susceptible, or in other words, diseased cucumber plants, germinated freely, and the germ-tubes were strongly chemotactic.

These experiments, coupled with others made on immune tomato plants, using spores of *Macrosporium tomato* for the purpose, prove that in future we shall be justified in defining an immune plant as an individual, in which the positive chemotactic substance, necessary for facilitating the entrance of the germ-tubes of a given parasitic fungus into its tissues, is absent.

The endeavour to produce immune races of economic plants has been much prosecuted of late years, and a certain amount of success has in some instances attended such endeavours; but apart from the fact that immune plants have constituted the basis of such experiments, the points aimed at have been the production of a more resistant cuticle, bloom on the leaves, &c.

Now, with clearer knowledge as to what constitutes immunity, it follows that the modifications required to attain this desired object are physiological rather than morphological; and the first step must necessarily be the determination of the exact constituent or constituents of the cell-sap, exercising a chemotactic influence over the germ-tubes of parasitic fungi.

MARSHALL WARD has demonstrated (3A), that the respective susceptibility or immunity of species of *Bromus*, to the attack of Uredospores of *Puccinia dispersa*, is not at all influenced by morphological characters. His summary on this point is as follows:—“*The capacity for infection, or for resistance to infection, is independent of the anatomical structure of the leaf, and must depend on some other internal factor or factors in the plant.*”

The method of procedure which has resulted in demonstrating the possibility of inducing saprophytic species of fungi to become parasites, or of educating a fungus already a parasite, to change its host-plant, is again only an extension of the system followed by MIYOSHI in studying chemotaxis, or as he writes it, “chemotropism.”

This author, among other experiments, sowed spores of *Penicillium glaucum* on the surface of a leaf of *Tradescantia discolor* into the tissues of which a 2-per-cent. solution of cane sugar had been injected. Not only did the germ-tubes of the *Penicillium* pass through the epidermis, but the mycelium spread vigorously and showed a tendency towards parasitism in the tissues of the leaf.

It is important to note that this tendency towards parasitism was manifested by *Penicillium* during the single experiment conducted by MIYOSHI. This evidence

suggested the idea that if the spores produced by a fungus showing a parasitic tendency, were again used for infecting a similar kind of host-plant, the incipient parasitism might gradually become intensified, and experiments about to be described proved this idea to be correct.

Experiments were conducted with the following species of fungi, which happened to be available at the time. *Trichothecium candidum*, *Torula herbarum*, *Stachybotryum lobulatum*, and *Stysanus stemonites*. These all belong to the Hyphomycetes and so far as is known are pure saprophytes, growing on decaying plants, damp paper, dung, &c. Spores of these fungi were first sown on perforated films of mica placed on gelatine containing 2 per cent. of a solution of cane sugar. The germ-tubes of *Stemonites* were not attracted by the sugar solution, hence this species was omitted from further experiments.

Six sowings of each of the three remaining species were made on living leaves still attached to the plant, *Begonia kewensis*, which had previously been injected with a 2-per-cent. solution of cane sugar in water. The spores were sown in a film of water on the surface of the leaf over the portions injected with sugar. An equal number of check sowings were made at the same time, on leaves not injected with a sugar solution.

Five days after sowing the spores, two out of the six patches infected with *Trichothecium candidum* each showed a few erect hyphæ, bearing spores, and microscopic examination showed that these hyphæ emerged from the interior of the plant through the stomata. These spores were at once sown on *Begonia* leaves injected with sugar solution as before, and this method of sowing spores produced by the preceding generation was repeated fifteen times. From four to five of the six patches sown produced spores during the last six batches of experiments, proving that the fungus was becoming more and more accustomed to its new environment.

When the quantity of spores produced from leaves injected with sugar solution became fairly abundant, some of these were sown on *Begonia* leaves that had not been injected with a solution of sugar; and from a sowing of the twelfth generation of spores produced on injected areas, but sown on a leaf not containing an injected chemotactic substance, I was delighted to see, the fourth day after sowing the spores, a small crop of sporophores bearing spores. This means that after twelve generations of the fungus, educated to grow in living *Begonia* by means of a chemotactic substance—a solution of cane sugar—the faculty of parasitism had been acquired for this particular host-plant.

Spores obtained from the thirteenth and fourteenth generations grown on injected areas showed more vigorous parasitism; whereas spores from the fifteenth and last crop of spores produced from injected areas, proved to be vigorously parasitic when sown in a film of pure water on *Begonia* leaves not injected with a solution of sugar.

The period of time required in this series of experiments for the conversion of a

saprophytic fungus into a true parasite was twelve weeks, and the number of generations of the fungus amounted to sixteen. The host-plant during the experiments was growing in a house where the day temperature ranged between 70° and 80° F., falling during the night to 60° F.

The actual proofs that the *Trichothecium* had become a true parasite on the *Begonia*, consisted in the presence of a copious development of mycelium in intercellular spaces of the leaf, and the occurrence of numerous short, special branches or haustoria in the cell cavities; and also the fact that specialised branches or sporophores, originating from the intercellular mycelium, passed through the stomata and produced fruit in the air. The portions of the leaf infested with the fungus eventually sunk below the general level of the surface, became brown, and the cells collapsed.

The check sowings of spores on *Begonia* leaves not injected, always ended without showing any appearance of parasitism, and never produced fruit; neither could I discover any traces of mycelium in the tissues of the leaf at these points where the spores were sown.

Returning to the results of the experiments made with the spores of *Stachybotryum lobulatum* and *Torula herbarum*. No signs of injury to the host-plant followed sowings of spores of the first-named, and no trace of mycelium was detected in the tissues, nevertheless the spores germinated readily in a hanging drop consisting of a 2-per-cent. solution of cane sugar. It is obvious that for some unknown reason, the germ-tubes of *Stachybotryum* could not effect an entrance into the *Begonia* leaf.

With *Torula herbarum* the case was very different; those areas of the leaf over which the spores were sown soon showed signs of internal disturbance; in fact, these symptoms were more pronounced than in the case of *Trichothecium*, and sections of the leaf showed a fair development of mycelium in the intercellular spaces. Nevertheless the fungus never produced fruit on the surface of the leaf over the infected areas, and this I consider as the most important evidence of parasitism. The results were as described above in twelve sowings, out of which seven showed indications of mycelium in the tissues. In one example where the appearance of the infected area indicated special activity of mycelium in the leaf, I tore a small slit in the epidermis with a fine needle, and at the expiration of two days a copious growth of spores was produced in the opening made in the epidermis.

These spores, as soon as their power to germinate had been determined, were sown on injected patches of *Begonia* leaves, but the result was as previously described, several of the patches showed evidence of the presence of living mycelium in the interior of the leaf, but no spores were developed.

Experiments made with another Hyphomycete, *Zygodesmus fuscus*, gave results similar to those of *Torula*; evidences of parasitism followed the sowing of the spores, but in no instance were spores produced. On the other hand *Cladosporium epiphyllum* spores, when sown on *Begonia* leaves under the conditions already

described, eventually became parasitic, and produced fruit on the surface of the leaf, the sporophores pushing through the stomata from the interior. In this instance, however, the parasitism was very weak, compared with that of *Trichothecium candidum*.

The reason why one species of saprophytic fungus can be induced to become parasitic on a given host-plant, whereas another species treated in every respect in a similar manner fails to do so, I have not been able as yet to determine. The two species that eventually became genuine parasites, *Trichothecium candidum* and *Cladosporium epiphyllum*, agree in having comparatively elongated sporophores, which grow erect from the prostrate vegetative mycelium. Now it is just possible that if one of these sporophores originated from mycelium located in the large intercellular space underlying a stoma, the innate tendency to grow erect might be sufficient to enable it to push through the stoma. On the other hand, the two species of fungi that failed to become parasitic, bear their spores on exceedingly short sporophores that do not grow erect or away from the mass of vegetative mycelium, hence their inability to reach the surface of the leaf. It would probably require a long experience to enable such fungi to form their spores in the substance of the leaf, and eventually rupture the epidermis for the purpose of spore dispersion, after the fashion followed by members of the Uredineæ, even if it could ever be acquired.

A further set of experiments conducted exactly on the lines already indicated, but using an infusion of cucumber leaves instead of a solution of cane sugar for injecting, proved that after twenty-one generations *Cercospora melonis*, an obligate parasite on plants belonging to the genus *Cucumis*, so far as is at present known, was trained to become a true parasite on *Oncidium bellatulum*, a plant belonging to the Orchidaceæ.

It will be observed that a greater number of generations, implying a longer period of time, was required to educate a fungus, already a parasite on one particular plant, to become parasitic on another plant belonging to a family widely separated from the one on which it was originally parasitic than to train a pure saprophyte to become a parasite. The only suggestion I can offer on this point is that the obligate parasite is much more dependent on one particular element for its development than is the case with a saprophyte. The explanation however, I am afraid, cannot be considered altogether satisfactory, inasmuch as at least many saprophytes are also obligate, or, in other words, are mainly if not entirely met with on one particular kind of nutritive pabulum.

It may be argued that the method of injection described above wounds the host-plant, and that the fungus grows as a saprophyte on the wounded portion of the leaf. The fact that the fungus experimented with under such conditions eventually becomes a parasite, whereas spores of the same fungus sown on leaves of the same plant, not injected with a chemotactic substance, are not capable of entering the

tissues, proves, I consider, that the injected substance, to which the fungus already responds, merely serves the purpose of enabling the fungus to become accustomed by degrees to a new nutritive and chemotactic substance present in the living tissues of the plant; when this acquirement on the part of the fungus is complete, parasitism results.

The experiments described above suggest the idea that an increase of virulence on the part of a parasite is possible by cultivation. This possibility, or even probability, I consider to be greatly influenced by circumstances. Last year the very destructive epidemic among cucumber plants, which resulted in a loss of over £20,000 to the growers of these plants in the south of England, was entirely due to the fungus called *Cercospora melonis*.

This spring a market gardener, whose cucumbers had suffered severely from the *Cercospora* last season, brought some cucumber plants to Kew, which he was afraid were attacked by the same disease. Examination showed that the plants were not attacked by *Cercospora melonis*, but by a fungus called *Dendryphium comosum*. The injury caused by the *Dendryphium* has now assumed the proportions of an epidemic in the cucumber houses of this particular market gardener, but is not recorded under such circumstances from elsewhere. Now *Dendryphium comosum* is a well-known saprophyte, growing on decaying vegetable matter, but its occurrence as a parasite has never before been recorded.

A careful examination as to the origin of the outbreak of the epidemic caused by *Dendryphium* revealed the fact that the young cucumber plants were growing in a mixture of two thirds loam and one-third of stable manure. Further, the portions of half-decomposed straw from the manure that projected above the surface of the soil, were covered with a copious growth of *Dendryphium*. The fungus had been introduced with the manure.

Dendryphium spores from the manure, when placed in a drop of water on the under surface of living cucumber leaves, produced the disease. The germ-tubes of the spores were positively chemotactic to a decoction of expressed juice from cucumber leaves, but failed to respond chemotactically to the expressed juice of twelve other plants selected at random and representing seven natural orders.

The above I consider to demonstrate the presence of a latent power—that of being positively chemotactic to a given substance or combination of substances—possessed by a saprophytic fungus. In the present instance the *Dendryphium* happened to be brought in contact with a living plant possessing the chemotactic influence required to enable the fungus to become a parasite.

A second illustration of extended parasitism, due entirely to opportunity, is afforded by *Puccinia malvacearum*. This fungus was first observed in Britain in 1873, on cultivated hollyhocks. Since that date the fungus has attacked all our indigenous malvaceous plants included in the three genera, *Malva*, *Althæa*, and *Lavatera*. It has also occurred on species of *Abutilon* in greenhouses.

It would be interesting to determine whether the forms of this fungus growing on different genera have become "biologic species" within 30 years.

The above conclusions are simply the reversion of experiments conducted by RAY (12), who in his studies on the biology of parasitism, considered the first important step to be that of educating obligate parasites to become saprophytes. This was accomplished by first growing such fungi in a decoction of the plant on which they were parasitic, followed by cultures in a decoction of a variety of the host-plant, then in that of allied species of plants; thus by a simultaneous process of weaning the fungus of its original food, and accustoming it to a new diet, such pronounced parasites as species of *Ustilago* and *Puccinia* were eventually trained to grow vigorously as pure saprophytes.

The experiments described above I consider to prove, what has previously only been assumed, that parasitism in fungi is an acquired habit.

In experimenting with comparatively large living plants the risk of contamination is great, and in spite of all precautions the cultures are too frequently ruined by the growth of saprophytic fungi such as *Mucor*, *Penicillium*, &c. Before injecting with the chemotactic solution the entire plant, soil, and plant-pot were thoroughly sprayed with a solution of permanganate of potassium. The plant-pot was placed on a sheet of glass covered with damp sterilised blotting paper and covered with a bell-jar also sterilised inside. Through a small opening at the top of the bell-jar water could be conveyed to the plant through a glass tube, and the nozzle of a small sprayer, of the kind used for fixing pencil drawings, could also be introduced, and the plant and inside of the bell-jar sprayed from time to time, with permanganate of potassium solution, which answered the double purpose of arresting the growth of stray fungi, and of keeping the atmosphere damp. As the spraying was directed from above, only the upper surface of the leaves was covered with the fungicide, and this did not interfere with the injected areas on the under surface of the leaves. The experiments described above were conducted in the Jodrell Laboratory, Royal Botanic Gardens, Kew.

In conclusion, it may be well to call attention to the parallelism between the germ-tubes of fungi and pollen-tubes. MIYOSHI (7A) has proved that pollen-tubes show marked positive and negative chemotactic properties; this may account for the behaviour of pollen when placed on the stigmas of flowers other than its own species, and may possibly be the determining factor in the production of hybrids. MARSHALL WARD (3B) gives an exceedingly interesting account "of some remarkable resemblances or coincidences, between the behaviour of these fungus-spores towards their host and, reciprocally of the host to the parasite, and that of the pollen and stigma the one towards the other."

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Summary.

1. The entrance of the germ-tubes of a parasitic fungus into the tissues of a living, healthy plant depends on the presence of some substance, in the cells of the host, attractive to the fungus. In other words, infection is due to positive chemotaxis.

2. A saprophytic fungus can be gradually educated to become an active parasite to a given host-plant, by means of introducing a substance positively chemotactic to the fungus into the tissues of the host. By similar means a parasitic fungus can be induced to become parasitic on a new host.

3. An immune plant signifies an individual of the same species as the one on which a given species of fungus is parasitic; but which, owing to the absence of the chemotactic substance in its tissues necessary to enable the germ-tubes of the fungus to penetrate, remains unattacked.

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